Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 1, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ASSESSING OF THE IMPACT OF HEMPSEED *JUBILEU* VARIETY ON PIG GROWTH PERFORMANCE, N METABOLISM, AND N_2O PREDICTION

Gabriel MIHĂILĂ[1], Mihaela HĂBEANU[2], Anca GHEORGHE[2], Nicoleta LEFTER[3], Livia VIDU[1], Carmen Georgeta NICOLAE[1], Monica MARIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Station for Sericulture Baneasa, Bucharest, 013685, Romania
[3]Animal Nutrition and Biotechnology Department, National Research-Development Institute for Biology and Animal Nutrition, Calea Bucuresti No. 1, Balotesti, 077015, Ilfov, Romania

Corresponding author email: mihaela.habeanu@scsbaneasa.ro

Abstract

Hempseed has an excellent antioxidant content and is a rich source of essential amino acids and n-3 fatty acids. The objective of this paper consists on evaluating the hempseed Jubileu variety effects on performance, nitrogen (N) balance. The prediction of nitrous oxide (N_2O) emissions was done as well. For 3 weeks of trial, a total of 15 pigs (39 kg ± 0.32) were randomly allocated individually in digestibility cages. Following a 7-day adaption period, there were two balancing periods where urine and feces were quantitatively collected. Two types of diet were used: Control group received a classical diet and Experimental group which received the same diet, supplemented with 5% hempseed. The growth parameter and carcass quality were not significantly altered by hempseed addition; however, a slight increased of ADG and feed conversion was noticed. Due to the significantly decreased of N concentration in the urine, TNO value was lower as well ($P<0.05$). A decline of N digestibility was noticed for HS group diet ($P<0.001$). Average value of N2O estimated was lower in HS fed group ($P = 0.03$). In conclusion, hempseed is a proteo-oleaginous source, which can be added in pigs' diet with beneficial effect on performance, N metabolism indicators and N_2O emissions.

***Key words**: digestibility, hempseed, nitrogen (N), nitrous oxide (N_2O), pigs.*

INTRODUCTION

A vital nutrient for pigs is nitrogen (N), required in very large quantities throughout the pig's life cycle, mainly for the synthesis of nucleotides, but also as a structural element of proteins. N is involved in many metabolic processes, but it is one of the most expensive nutrients for pigs and can have a negative impact on the environment. A large amount of ingested nitrogen is eliminated through excretion (2/3 of ingested N) (Millet et al., 2018, Hăbeanu et al., 2020), in the form of nitrous oxide (N_2O) and ammonia (NH_3), compounds associated with a negative impact on the environment. The global warming potential of N_2O is 298 times higher than that of carbon dioxide (CO_2). N_2O is formed as a result of nitrification and denitrification processes.

As a result of drought and other less controllable phenomena, the accessibility and price of some fodder resources changes, and implicitly there is the need to evaluate the nutritional, productive potential and the impact on the environment of any plant resources and/or by-products available (Dmytrotsa et al., 2023).

Current approaches have suggested that oil-rich forages and their by-products containing poly-unsaturated fatty acids (PUFA) in a high concentration could decrease enteric CH_4 generation in ruminants (Ebeid et al., 2020) and monogastrics (Hăbeanu et al., 2022). However, adding oil to feed also reduced dry matter intake, although this strategy can be relatively expensive. Nutritional solutions have been identified and developed for the effective utilization of some less studied plant resources to complement or replace part of the classic pig resources (corn and soybean meal). For example, in pigs, mustard cakes associated with those from grape seeds were tested (Hăbeanu et al., 2022), peas, guar flour (Mihăilă et al., 2023; Hăbeanu et al. 2020) They were established as potential predictors of CH_4 enteric volatile fatty acids that are in close correlation with the fiber level of the food and related to it with the microbial population (Hăbeanu et al., 2022). An increased level of fiber through the addition of a mixture of grape cakes and mustard, can lead to a slight decline in performance, but at the environmental level the effects are positive, in the sense that the amount of excreted nitrogen is reduced (by approx. 6% per the background of the higher level of fiber and its fractions). The same trend was observed regarding N_2O which decreases by approx. 5.5% in animals fed with mustard cakes (Hăbeanu et al., 2021). In the same species, although peas and linseed have valuable nutritional potential and can replace part of soybean meal, due to a higher intake of some carbohydrate fractions, a slight decline in growth performance is reached. However, the mixture between peas and flax seeds contributed to its cancellation (Hăbeanu et al., 2020). Based on the above, it can be hypothesized that feed with the addition of oleo-proteins and by-products with a different structure can represent an effective method of reducing excreted nitrogen with implicit effects on the environment.

Prohibited for a long time, but re-included According to the EFSA Regulation (2015) in the list of feed raw materials that can be used in animal feed, hemp returns to the center of attention of specialists due to its nutritional composition of interest for animal husbandry. Data in the scientific literature are lacking or incomplete regarding the impact hemp seed has on pig performance and emissions.

Hemp is one of the oldest industrial plants. The *Jubileu* hemp variety was approved in 2012. Hemp seeds contain approximately 30% oil, and 80% of this oil is made up of unsaturated essential fatty acids, which are not synthesized by the body, and 31% easily digestible proteins that can supplement or replace other protein sources. Hemp seed protein contains all 21 known amino acids, including the 8 essential amino acids that the body cannot produce, namely leucine, lysine, methionine, phenylalanine, tryptophan, valine and threonine. According to Bälter et al. (2017), feed ingredients of animal origin emit more GHGs than raw materials of plant origin, it seems that sows have an even greater impact on GHG amounts, especially enteric CH_4 (E-CH4) and from manure M-CH_4 and N_2O (House, 2010; Hăbeanu et al., 2021).

Reducing GHG emissions through pig feeding and manure management remains a major issue for intensive pig farmers, as the microbial process of N cycling and manure C content, as well as storage and treatment time, determine the amount of N_2O emitted during storage (House, 2010; Bälter, 2017).

This study was conducted on pigs to evaluate the effects of *Jubileu* hemp seed on performance and nitrogen metabolism and N_2O prediction based on indicators derived from nitrogen balance experiments.

MATERIALS AND METHODS

1. Ethical procedure

The balance experiment of N was carried out at INCDBNA Balotești, and was carried out in accordance with the experimental protocol authorized by the Institute's Ethics Committee and in compliance with the provisions of Law no. 199/2018 and from EU Directive 2010/63/EU on animal research. *Jubileu* hemp seeds were supplied by SCDA Secuieni and are characterized by a high nutritional value and a similar cost compared to other protein sources.

2. Experimental scheme and treatment

The nitrogen balance experiment was carried out for 3 weeks (1 week of acclimatization and 2 weeks of balance) on a number of 10 Topigs hybrid male pigs [♀ Large White x Hybride (Large White x Pietrain) ♂ × Talent, mainly Duroc] growing - fattening. The mean initial weight was 39.65 kg ± 0.53. Animals were randomly assigned to two groups (Control and experimental), each with five repetitions. The weight of the animals was recorded at the beginning and at the end of the experiment. Individual metabolism cages (1.2 × 1.5 × 1 m) were used. The temperature during the experiment was 22 ± 1°C.

Feed was given ad libitum twice a day (08.00 h and 15.00 h) and water *ad libitum*.

The hemp, the *Jubileu* variety, was supplied by SCD Secuieni. To evaluate growth performance, nitrogen balance and N_2O prediction, *Jubileu* hemp seeds were subjected to laboratory analysis for the determination of crude protein (CP), ether extract (EE), fiber (CF), amino acids and minerals. The composition and nutritional characteristics of the *Jubileu* hemp seeds used in compound feed recipes administered to pigs are shown in Table 1.

Table 1. Nutritional characteristics of *Jubileu* hemp seeds

Jubileu* hemp seeds	**(%)**
C18:3n-3	17.06
Ratio n-6:n-3	3.20
Dry matter (DM)	89.67
Protein (CP)	21.26
Ether extract (EE)	27.70
Fiber	28.82

*C18:3n-3 – α-linolenic polyunsaturated fatty acid

The composition and nutritional characteristics of the compound feed recipes used in the feed of the control group and the experimental group with an intake of 5% *Jubileu* hemp seeds (HS) are presented in Table 2.

Table 2. Structure of compound feed recipes for grow - fattening pigs (%)

Ingredients (%)	Control group (C)	Experimental group (HS)
Maize	62.9	60.02
Rice flour	10	10
Hemp *(Jubileu)*	0	5
Soybean meal (44%)	15	12
Sunflower meal	8	9
DL-methionine	0.01	0.01
L-Lysine-HCl	0.28	0.32
Calcium carbonate	1.65	1.7
Monocalcium phosphate	0.65	0.45
Salt	0.4	0.4
Choline premix	0.1	0.1
Vitamin and trace mineral premix (P3+4)**	1	1
Nutritional characteristics (g x kg^{-1} as feed bases)		
DM	87.80	87.99
Metabolizable energy (ME, Mj x kg^{-1}))	13.64	13.78
Crude protein (CP)	15.21	15.12
Ether extract (EE)	3.46	4.78
Fiber	4.05	4.72
Lys	0.91	0.91
Lys d	0.81	0.82
Met +Cys	0.57	0.57
Met +Cys d	0.51	0.51
Calcium (Ca)	0.89	0.87
Phosphorus (P)	0.51	0.50

*Abbreviation: dry matter (DM), lysine (Lys), methionine + cysteine (Met + Cys), digestible (d).
**The vitamin-mineral premix introduced in the compound feed is specific for the age and weight category and provided per kg of feed: 6000 IU vitamin A; 800 IU vitamin D3; 20 IU vitamin E; 1 mg vitamin K3; 1 mg vitamin B1; 3.04 mg vitamin B2; 10 mg vitamin B3; 6.3 mg vitamin B5; 1.5 mg vitamin B6; 0.03 mg vitamin B7; 0.3 mg vitamin B9; 0.02 mg vitamin B12; 30 mg Mn; 80 mg Fe; 25 mg Cu; 100 mg Zn; 0.22 mg I; 0.22 mg Se; 0.3 mg Co; 60 mg antioxidant.

The compound feed recipes contain the energy and protein requirements related to the growth category. *Jubileu* hemp seeds come with an increased intake of CP. To obtain the same level of CP, the level of sunflower meal was slightly increased and the level of soybean meal decreased. To ensure an optimal level of ME, maize adjustments were made at different levels. The crystalline amino acids DL-methionine and L-lysine-HCl were included in the developed recipes to cover the requirements for the two recipes, as well as calcium carbonate and monocalcium phosphate to provide Ca and P requirements.
The level of fiber was higher in the diet of the experimental group (HS) compared to the diet of the control group (C). Food was provided twice a day at 08:00 and 14:00 and water was available *ad libitum*.

3. Measurement and sampling

3.1. Growth performance and carcass quality

The body weight (BW) of the pigs was recorded on an electronic scale at the beginning and end of the digestibility test. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated based on BW (Broucek, 2017), equations used to calculate Kleiber ratio (KR), relative growth rate (RGR, %) based on ADG and Metabolic BW ($MBW^{0.75}$) on the one hand and BW and age on the other.
Carcass characteristics (backfat thickness, *Longissimus dorsi* area and lean meat percentage) were determined on the left side using the PIGLOG 105 ultrasound machine (SFK-Technology, Denmark) equipped with a meat percentage evaluation formula: Y= 64.39-0.28 Fat-1+0.14 LD muscle thickness-0.55 Fat-2, where LD is the *Longissimus dorsi* muscle. Fat-1 was measured 7 cm laterally behind the last rib from the middle dorsal line, while fat-2 was measured 10 cm from the last rib to the cranial section and 7 cm lateral to the middle dorsal line.

3.2. Nitrogen balance

During the two periods of nitrogen balance (4 days per week), urine and faeces samples were collected, at 08.00 h-08.30 h. Fecal samples collected from each animal were mixed and homogenized for each batch, and 10% of the amount obtained was frozen at -18°C for analysis (according to the experimental protocol described by Hăbeanu et al. (2019). The volume of urine was recorded daily and 10% was separated and stored at -18°C for analysis. To lower the pH and conserve nitrogen, H_2SO_4 25% concentration was used in each urine container. Pig faecal samples of 0.4 g were weighed with an accuracy of $\pm 2 \times 10^{-4}$ g.

3.3. Chemical analyses

The chemical composition of *Jubileu* hemp seeds, of NC administered during the experiment, was determined in the Chemistry Laboratory of IBNA Balotești. The analyzed samples were collected in duplicate, for determinations of DM, CP, EE, crude fiber (CF), in accordance with the recommendations of Commission Regulation (EC) no. 152 (JOUE, 2009). The traditional semi-automatic Kjeldahl technique using Kjeltek auto 1030 – Tecator (Standard SR EN ISO 5983-2:2009 AOAC 2001.11) was used for CP determination. CF extraction was performed using an intermediate filtration method (Standard SR EN ISO 6865:2002).

The semi-automatic Kjeldahl method (Kjeltec Auto 1030 Analyzer, Hillerod, Denmark) was used to determine the N content of the excreta. The samples were subjected to distillation and titration. Class A glassware was used for decanting, dilution and storage. All reagents used were supplied by Merck (Darmstadt, Germany). Stock solutions traceable to the National Institute of Standards and Technology (NIST) standard reference material (SRM) were used for calibration. Nitrogen retained (NR), total nitrogen output (TNO) and nitrogen digestibility were determined by measuring N intake (based on DM) and N excretion according to the methods described by Hăbeanu et al. (2019), using the equations described by Hlatini et al. (2020).

Determinations of fatty acids were performed from the fodder samples: CI4:0 (myristic); CI6:0 (palmitic); CI8:0 (stearic); CI8:1 cis-9 (oleic); CI8:2n-6 (linoleic); CI8:3n-3 (α-linoleic); CI8:4n-3 (octadecatetraenoic); C2O:4n-6 (arachidonic) and C22:I-9 (uric);

All determinations were performed in duplicate by high-performance liquid chromatography using a Surveyor Plus Thermo Electron HPLC (Waltham, MA, USA) equipped with a HyperSil BDS C18 silica column (Thermo Electron, Waltham, MA, USA). Dimensions: 250 mm × 4.6 mm × 5 µm, according to the methods described in the research papers by Vărzaru et al. (2013), Hăbeanu et al. (2019), and Gheorghe et al. (2020).

3.4. Calculations

3.4.1. The coefficient of total tract apparent (CTTAD), coefficient of apparent metabolizability (CAM), biological value of protein (BVP) and net protein utilization (NPU) were calculated using the following equations:

CTTAD = (N intake – faecal N output)/N intake (1)

CAM = N intake − N fecal output − N urinary output/N intake (2)

BVP = N retained/N digested (3)

NPU = N retained/ Nintake (4)

3.4.2. Prediction of N_2O emission

Equations based on input data (recorded at the beginning of the experiment) and output data recorded at the end of the experiment were used to predict N_2O. The equations have been established in IPCC (2006, 2019) and earlier work. The TNO assessed in our study was integrated into the N_2O prediction equation given by Philippe & Nick (2014): N_2O = TNO x 0.2/100 x 44/28. The conversion factor of excreted N was 0.2 to N_2O (IPCC, 2006), for manure storage spaces under animal housing and 44/28 is the ratio between the molar mass of N_2O and that of N. The N_2O value of 298 - times greater global warming potential than CO_2 was shown in Eq-CO_2.

3.4.3. Statistical analyses

IBM SPSS (2011, version 20) is the statistical program for the analysis of recorded experimental data. Animals were assigned to two experimental groups in a completely randomized experimental design.

ANOVA test was used to check the significance of the data. The impact of diet is considered statistically significant if the P value is less than 0.05. Repetition values were not included in the study if the P value was greater than 0.05. The Pearson test was used to determine and measure correlation.

EvaPig® software, version 2.0.3.2 (2020), created by the French National Institute of Agricultural Research, Metex Nvistago and the

French Association of Animal Husbandry was used to develop compound feed recipes.

RESULTS AND DISCUSSIONS

1. Chemical composition of the analyzed feed ingredients

Following the laboratory determinations regarding the chemical composition of the feed ingredients analyzed (Table 3), *Jubileu* hemp seeds stand out for their high protein content - CP analyzed (21.26%), representing almost half the value of soybean meal, EE (27.70%) was higher than in the meal due to the fact that the fat is extracted from the meal, and the concentration in essential amino acids for pigs was 0.959% Lyz., 0.832% Met., 0.347% Cys.

Although lysine is at a lower level compared to that of soybean meal, which determined the addition of synthetic lysine to the feed at a level 1.14 times higher than in the control group, Met + Cys have close average values in the two ingredients.

However, the level of lysine, but also of Met + Cys, in *Jubileu* hemp is higher than that reported by House et al. (2010), respectively an average of 0.860 for Lys and 0.97 for Met + Cys.

Table 3. Comparative chemical composition of soybean meal and hemp seed

Specification (%)	Soybean meal	*Jubileu* hemp
Composition		
DM	87.74	89.67
CP	44.0	21.26
EE	1.69	27.70
Fiber	6.29	28.82
Amino acids (AA)		
Lysine	2.75	0.959
Methionine	0.64	0.832
Cysteine	0.67	0.347
Met. + Cyst.	1.31	1.179
Minerals		
Ca	0.20	0.09
P	0.60	0.93

2. Growth performance and carcass quality

The mean values and standard error of the mean (SEM) of the results obtained by determinations on animals regarding growth performance are presented in Table 4, and carcass quality is presented in Figure 1.

Table 4. Mean and SEM of consumption and performance for growing-fattening pigs

Specification*	Control group	Experimental group	SEM	P**
Consumption of compound feed (kg/day)	2.77	2.51	0.06	0.30
Consumption of DM (kg/day)	2.43	2.21	0.06	0.30
IBW (kg)	40.10	37.30	0.24	0.60
FBW (kg)	67.0	65.6	0.78	0.16
Average daily gain (ADG) (kg/day)	1.05	1.12	0.03	0.10
Kleiber ratio	5.28	5.78	0.09	0.08
Feed conversion ratio (FCR)	0.38	0.45	0.01	2.77
CAM	0.46	0.44	0	0.9
CTTD	0.88	0.86	0	0.88

*Abbreviation: dry matter (DM); initial body weight (IBW); final body weight (FBW); coefficient of apparent metabolizability (CAM); coefficient of total tract apparent (CTTAD). Feed conversion ratio was calculated as the ratio of ADG to the daily consumption of compound feed.
**P>0.05 insignificant differences.

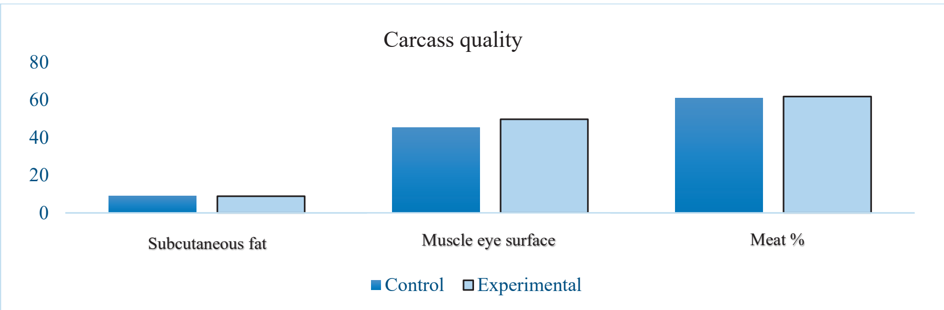

Figure 1. Means and SEM of carcass parameters as effect of 5% hemp seed addition; P>0.05 non-significant differences between batches. To determine carcass traits, measurements were performed on live animals with the Pig log 105 apparatus

As the obtained data show, body weight, average daily gain, food consumption and feed conversion ratio were not significantly affected in the experimental group, which received the addition of hemp seeds in the food. On the contrary, the average daily gain was 6% higher in the case of the experimental group, and the feed conversion increased by 18.42%. Coefficient of apparent metabolizability (CAM) and coefficient of total tract apparent (CTTD) were similar (P>0.05).

Subcutaneous fat in the experimental group was 3.27% lower compared to the control group, and the muscle eye surface in the experimental group was 9.73% higher compared to the control group, but without the differences between the means being statistically significantly different. The same aspect was observed in the case of the percentage of meat, i.e. a higher share of it by 1.49% in the experimental group.

3. Nitrogen balance and N_2O prediction

3.1. Nitrogen metabolism

Table 5 shows the mean experimental data and SEM for the nitrogen balance indicators, as well as the value estimated by equations for predicting the amount of N_2O.

A significant decrease in urinary nitrogen by 12.49% was observed, which led to a decrease in total nitrogen output (TNO) content by 10.86% for pigs fed with the addition of 5% *Jubileu* hemp seeds in the feed (P<0.05). There was a decrease in the percentage of N retained (NR) by 10.12% (P = 0.016), due to a lower N digestibility coefficient by 1.39% in pigs from the experimental group. The net protein utilization (NPU) value was similar.

As a result of the correlation between the balance parameters and N_2O, a significant reduction of the latter was recorded by 10.81%, although, if we refer to ingested N, ingested DM and the average daily gain register an insignificant increase.

3.2. Correlations

To establish the influence of various dietary factors on N metabolism parameters, the Pearson correlation shown in Table 6 was estimated. As expected, DM and N consumption have a close positive correlation with TNO, NR and implicitly with N_2O (P<0.0001). Average daily gain is not correlated with N and its derivatives resulting from metabolic processes.

Table 5. Mean and SEM values for nitrogen balance indicators and N_2O prediction

Specification*	Control group	Experimental group	SEM	P**
N balance				
Urinary N (g/day)	28.45	25.29	0.70	0.02
Fecal N (g/day)	7.63	7.27	0.12	0.13
TNO (g/day)	36.08	32.56	0.82	0.03
NR (g/day)	31.22	28.17	0.64	0.016
N digestibility (%)	88.58	87.37	0.16	<0.001
NPU (g/day)	46.45	46.46	0	0.9
N_2O prediction				
N_2O (g CO_2 Eq/day)	33.80	30.50	0.76	0.031
N_2O / ingested N (g CO_2 Eq/day)	0.59	0.64	0.01	0.11
N_2O /DMI (g CO_2 Eq/day)	16.49	17.71	0.46	0.14
N_2O/ ADG (g CO_2 Eq/day)	38.59	35.02	0.10	0.10

*TNO - total nitrogen output; nitrogen retained (NR), NPU - net protein utilization
**P>0.05 insignificant differences; P<0.001 very significant differences

Table 6. Pearson correlation between input data and indicators of nitrogen metabolism

Specification*		DM consumption (kg/day)	N consumption (g/head/day)	AVG (g/day)	N_2O (g CO_2 Eq/day)
TNO	R	1	1	-0.002	0.632
	P	<0.0001	<0.0001	0.990	<0.0001
NR	R	0.99	1	-0.011	0.620
	P	<0.0001	<0.0001	0.941	<0.0001
N_2O	R	0.632	0.631	0.119	1
	P	<0.0001	<0.0001	0.409	

*r= correlation
P>0.05 insignificant differences; P<0.001 very significant differences.

Discussions

Nutritional options are evaluated as tools to effectively reduce pollutant emissions. Respecting the nutritional requirements of the animals, using balanced feed recipes against the background of the use of lesser-known feed resources, can contribute to maintaining performance. Through this study, we aimed to deepen the knowledge regarding the effects of the addition of *Jubileu* hemp seed improvement in the feed of growing-fattening pigs on growth performance and nitrogen metabolism.

Hemp is a variety of *Cannabis sativa*, attested since ancient times on the territory of our country. Hemp culture is profitable because it is exploited in multiple ways (textiles, seeds and meals, oils). Hemp seeds contain approximately 30% oil, characterized by a valuable profile in n-3 FA, antioxidant properties and 31% easily digestible proteins (Hăbeanu et al., 2018). Hemp protein has high biological value due to its excellent amino acid profile.

As mentioned by Chen et al. (2023), hemp seed protein is becoming an important alternative source of vegetable proteins in food and as nutraceuticals in industry for its high nutritional values, processing ability and as a homologue with other medicinal plants. With changing dietary habits and increasing demand for natural food ingredients, the need to use hemp seed protein is expected to grow rapidly. However, to fully utilize the competitive advantages of hemp seed protein a number of key issues must be resolved for its promotion, namely: availability and price, primary and secondary effects on animal and human health, anti-nutritional factors, also active biocompounds present in this resource that could diversify the possibilities of use and, last but not least, the quantification of the effects on the environment through its use in animal feed.

Jubileu hemp is an improved variety obtained at SCDA Secuieni, Romania, monoecious, approved for seed and oil.

In the literature, to the best of our knowledge, there is no information on the effects of hemp seeds on N_2O emission. The high level of essential fatty acids for health, especially n-3, was an argument used in most studies to evaluate the influence of hemp (seeds or meal, oil) on meat quality or immune response and oxidative stress (Vodolazska & Lauridsen, 2020; Hăbeanu et al., 2018a,b; Palade et al., 2019; Li et al., 2022). Our previous studies considered the impact on N and its derivatives by using as feed a mixture between mustard cakes and grape cakes (Hăbeanu et al., 2021), or *Tudor* pea grains compared with *Lirina* flax and soybean meal (Hăbeanu et al., 2020) or of guar flour (Mihăilă et al., 2023). In 2010, House and in 2011, Presto et al. determined the digestibility of hemp protein and amino acids, demonstrating that hemp seed protein has highly digestible value either in its native form or as hemp seed meal, which these are also confirmed in our study.

According to the studies conducted by Ahmed et al. (2022), hemp proves competence in the search for new sustainable resources because it is naturally resistant to diseases and pests, conserves water, degrades quickly and produces environmentally friendly industrial products such as biodiesel, bio-composite, paper, textile, and so on. Hemp biofuel could be an excellent alternative to petroleum-based fuel to produce heat and power for transportation and industrial sectors. Hemp would break new ground by harnessing it in the paper industry using its advantage of higher yield and more recyclability of hemp paper than wood.

Lanzoni et al. (2024) showed that the inclusion of hemp-based products in the diet of monogastric animals led to different results depending on the species. The use of these products requires further investigation, particularly in pigs and broilers, due to limited studies. However, for laying hens, for example, the greater number of scientific studies have made it possible to identify and confirm hemp as an effective and safe source, capable of positively modulating animal health and performance, simultaneously enhancing the nutritional and functional profile of eggs. However, the inclusion of hemp in food requires further evaluation. Practically, in the literature there were no data regarding the impact of hemp on GHG emissions, with an emphasis on N_2O.

In 2018, GHG emissions from agriculture amounted to 17.5 million tonnes of CO_2 equivalent, representing 3.9% of the total EU GHG emissions from agriculture. Total GHG emissions generated by agriculture decreased between 1990 and 2018 by 44.3% (-29.3% in the EU) and ammonia emissions decreased (by

4%36) in the same period, this justifies due to the reduction of livestock numbers, but also due to investments in new technologies used in animal husbandry.

In 2016, methane (CH_4) and N_2O emissions reported per hectare (exploited agricultural area) were 1.29 kilotons CO_2 equivalent/1 000 ha, being among the lowest in the EU. Nitrogen management is an important part of the activities carried out on livestock farms and an important part of the process of estimating and reducing pollutant emissions.

In the present study, hemp seed did not affect growth performance and carcass quality in growing-fattening pigs, although a slight increase in ADG and feed conversion was observed. Our data are comparable to those obtained in 2018 by Hăbeanu et al.

Due to the significant decrease in the N concentration in the urine, the TNO value was also lower ($P<0.05$). A decrease in N digestibility was observed for the hemp seed-based feed group ($P<0.001$). In our study, the mean value of estimated N_2O was lower in the group fed with hemp ($P = 0.03$).

The N_2O level was higher than that reported by Hăbeanu et al. (2020) using soybean meal compared to peas and flax, or in 2021 by Hăbeanu et al., when they used mustard cakes and grape cakes in growing pigs. The consumption of DM and implicitly of N is correlated with indicators of nitrogen metabolism and implicitly with the emission of N_2O. Although the combined feed recipes were balanced, the mechanisms underlying the recorded impact differences should be sought in the depth of the physiological processes.

CONCLUSIONS

Hemp seeds are a proteo-oleaginous source that can be added to pig diets with a beneficial effect on productive performance, causing a slight increase in average daily gain and feed conversion.

The addition of hemp seeds in the compound feed recipes administered to pigs determined a decrease in the concentration of nitrogen in the urine and a low value of N_2O emissions.

ACKNOWLEDGEMENTS

This research paper is part of the doctoral thesis and was realized with the support of the Faculty of Animal Productions Engineering and Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest. This research was carried out with the support of the Ministry of Research, Innovation and Digitalization, Project Nucleus (23-20.04.01).

REFERENCES

Ahmed, A.T.M.F., Islam, M.F., Mahmud, M.S., Sarker M.E., & Islam, M.R. (2022). Hemp as a potential raw material toward a sustainable world: A review. *Heliyon*, 8. e08753.

Bälter, K., Sjörs, C., Sjölander, A., Gardner, C., Hedenus, F., & Tillande, A. (2017). Is a diet low in greenhouse gas emissions a nutritious diet? – Analyses of self-selected diets in the LifeGene study. *Archives of Public Health,* 75, 17. doi: 10.1186/s13690-017-0185-9.

Chen, H., Xua, B., Wang, Y., Li, W., He, D., Zhange, Y., Zhang, X., & Xing, X. (2023). Emerging natural hemp seed proteins and their functions for nutraceutical application. *Food Science and Human Wellness*, 12, 929-941.

Dmytrotsa, A., Vovk, S., Vorobel, M., & Klym, O. (2023). Optimization of indoor microclimate parameters is an important factor in stimulating metabolism in the body and increasing pig productivity. *Scientific Papers. Series D. Animal Science, LXVI*(2), 289-293.

Ebeid, H.M., Li, M., Kholif, A.E., Faiz-Ul, H., Yang, C., Peng, L., & Liang, X. (2020). Moringa Oleifera oil modulates rumen microflora to mediate in vitro fermentation kinetics and methanogenesis in total mix rations. *Current Microbiology, 77*(7), 1271–1282. doi:10.1007/s00284-020-01935-2.

Gheorghe, A., Lefter, N.A., Idriceanu, L., Ropotă, M., & Hăbeanu, M. (2020). Effects of dietary extruded linseed and *Lactobacillus acidophilus* on growth performance, carcass traits, plasma lipoprotein response, and caecal bacterial populations in broiler chicks. Ital. *J. Anim. Sci*., 19, 822–832.

House, J.D., Neufeld, J., & Leson, G. (2010). Evaluating the quality of protein from hemp seed (*Cannabis sativa* L.) products through the use of the protein digestibility corrected amino acid score method. *Journal of Agricultural and Food Chemistry*, 58, 11801-11807.

Hlatini, V.A., Ncobela, C.N., & Chimonyo, M. (2020). Nitrogen balance response to varying levels of dietary protein in slow-growing Windsnyer pigs. *S. Afr. J. Anim. Sci.*, 50, 644–653.

Hăbeanu, M., Gheorghe, A, Lefter, N.A., Untea, A, Idriceanu, L., & Ranta, M.F. (2020). Assessment of certain nitrogen metabolism indicators, enteric CH_4 and CO_2 emitted through manure related to different diets in barrow. *Archiva Zootechnica*, 23, 2, 129-142.

Hăbeanu, M., Lefter, N.A., Toma, S.M., Idriceanu, L., Gheorghe, A., & Surdu, I. (2021). Nitrous oxide prediction in manure from pigs given mustard x grapeseed oil cakes as a replacement for sunflower meal. *Archiva Zootechnica, 24*(2), 47-57, 2021, doi: 10,2478/azibna-2021-0012.

Hăbeanu, M., Lefter, N.A., Toma, S.M., Dumitru, M., Cismileanu, A., Surdu, I., Gheorghe, A., Dragomir, C., & Untea, A. (2022). Changes in ileum and cecum volatile fatty acids and their relationship with microflora and enteric methane in pigs fed different fiber level. *Agriculture*, 12, 451, https://doi,org/10,3390/ agriculture120404.

Hăbeanu, M., Lefter, N.A., Gheorghe, A., Untea, A., Ropotă, M., Grigore, D.M., Vărzaru, I., & Toma, S.M. (2019). Evaluation of Performance, Nitrogen Metabolism and Tissue Composition in Barrows Fed an n-3 PUFA-Rich Diet. *Animals, 9*(5), 234. doi.org/10.3390/ani9050234.

Habeanu, M., Gheorghe, A., Surdu, I., Chedea, V.S., & Beia, I. (2018a). N-3 PUFA-enriched hemp seed diet modifies beneficially sow milk composition and pigliets' performances. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 63, 40–45.

Hăbeanu, M., Gheorghe, A. Ropotă, M., Toma, S.M., & Marin, M. (2018b). Changes in fatty acids composition of longissimus dorsi muscle, brain, liver and heartas effect to hempseed addition in pigs feeding. *AgroLife Scientific Journal*, *7*(2), 61-67.

IPCC (2006, 2019). *Guidelines for National Greenhouse Gas Inventories.* Available online: https://www.ipcc-nggip.iges.or.jp/public/20 06gl/.

Lanzoni, D., Skrivanova, E. Pinotti, L., Rebucci, R., Baldi, A., & Giromini, C. (2024). Review: Nutritional aspects of hemp-based products and their effects on health and performance of monogastric animals. *Animal,* 18, 10105. doi.org/10.1016/ j.animal.2023.10105

Li, Q., Yang, S., Chen, F., Guan, W., & Zhang, S. (2022). Nutritional strategies to alleviate oxidative stress in sows. *Animal Nutrition*, 9, 60e71.

Mihăila, G., Habeanu, M., Lefter, N., Gheorghe, A., Dumitru, M., Marin, I., Vidu, L., Nicolae, C.G., Popa, D., & Marin, M. (2023). Influence of Guar Meal from Pig Compound Feed on Productive Performance, Nitrogen Metabolism, and Greenhouse Gas Emissions. *Agriculture*, 13, 2156. https://doi.org/10.3390/ agriculture13112156.

Millet, S., Aluwé, M.A., Broeke, V.D., Leen, F., De Boever, J., & De Campeneere, S. (2018). Review: Pork production with maximal nitrogen efficiency. *Animals, 12*(5), 1060–1067. doi:10.1017/S1751731117002610.

Palade, L.M., Hăbeanu, M., Marin, D.E., Chedea, V.S., Pistol, G.C., Grosu, I., Gheorghe, A., Ropotă, M., & Taranu, I. (2019). Effect of dietary hemp seed on oxidative status in sows during late gestation and lactation and their offspring. *Animals, 9*(4), 194. doi: 10.3390/ani9040194.

Philippe, F.X., & Nicks, B. (2014). Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. *Agric. Ecosyst. Environ.,* 199, e10–e25.

The European Food Safety Authority (EFSA) (2015). *Scientific Opinion on the risks for human health related to the presence of tetrahydrocannabinol (THC) in milk and other food of animal origin. EFSA Panel on Contaminants in the Food Chain (CONTAM),* 13(6), 4141.

Varzaru, I., Untea, A.E., Martura, T., Olteanu, M., Panaite, T.D., Schitea, M., & Van, I. (2013). Development and validation of an RP-HPLC method for methionine, cystine and lysine separation and determination in corn samples. *Rev. Chim*., 64, 673–679.

Vodolazska, D, & Lauridsen, C. (2020). Effects of dietary hemp seed oil to sows on fatty acid profiles, nutritional and immune status of piglets. *J. Anim. Sci. Biotechnol.,* 18, 11, 28. doi: 10.1186/s40104-020-0429-3.